NOTES ON THE PHYSIOLOGY OF FUCUS SPERMATOZOIDS.

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The notes here presented are of work conducted at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1912 and 1913 at the suggestion of Dr. B. M. Duggar, to whose kindness I also owe the opportunity and pleasure of working there.

Strasburger (1) states that spermatozoids of *Fucus*, passing at a distance of one or even two diameters from the egg, turn from their path and rush toward the egg. This attraction, he says, is a chemical one and is due to some substance secreted by the egg which conditions the direction of motion of the spermatozoids. Strasburger also states that healthy spermatozoids are strongly negatively phototactic. These two phenomena of phototaxy and chemotaxy are used by Strasburger as a basis upon which to account for the meeting of the spermatozoids and the ova as follows:

The ova have a density greater than water and sink. The spermatozoids, as they are negatively phototactic, swim downward bringing them into the region where the chemotactic influence of the eggs is sufficient to complete the union of the spermatozoids with the ova. Strasburger made no attempt to discover the chemotactic agent.

Bordet (2) also attempted an explanation of the same problem. He filled a capillary tube with crushed ova, immersed the tube in a drop of sea water containing *Fucus* spermatozoids, and watched for evidences of attraction, but found none. From this he concludes that the ova exert no chemotactic influence on the spermatozoids. He observed, however, that the spermatozoids were very sensitive to contact, clinging with one cilium to the glass slide or any solid object as, for example, a capillary tube. Bordet also states that *Fucus* spermatozoids are not phototactic,

being neither attracted nor repelled by light. He believes the meeting of the eggs and spermatozoids is due to chance.

In brief, Strasburger states that *Fucus* spermatozoids are negatively phototactic and are chemotactic to the ova. Bordet asserts that they are not phototactic and that there is no chemotaxy.

Material and Methods.

The diœcious form, *Fucus vesiculosus*, was used in the experiments.

To secure spermatozoids or ova for the investigation the methods described by Strasburger (1) were followed.

Inasmuch as fresh material could not be secured daily, some method of keeping the *Fucus* in good condition was desired. Sinking the material in the sea was found to yield active spermatozoids for only a day or two after bringing it in from its habitat. *Fucus* plants wrapped in towels wetted with sea water and kept in an ice chest yield active spermatozoids for at least five days after being brought in. The antheridia from fruiting tips kept in sea water in the ice chest for the same length of time were exuded on drying, but the spermatozoids were inactive and lay inert in the antheridia. This difference between the two treatments is probably due to differences in oxygen supply. Strasburger advises shipping fruiting *Fucus*, desired for active spermatozoids and living oögonia, in large amounts of sea water, but it appears that wrapping the *Fucus* in wet cloths and icing it would be preferable.

Experiment on Chemotaxy.

In investigating chemotaxy Pfeffer's capillary tube method was used. The capillary tubes mounted in a drop of sea water containing the spermatozoids were examined under the microscope for about three quarters of an hour for change in direction of motion of the individuals as they passed by the mouth of the capillary tube and for collection of the spermatozoids in the tube as described by Pfeffer and others for various Pteridophytes. The majority of the substances, on account of the strong negative phototaxy of the spermatozoids, were also tested by placing the mounts in the dark and examining them after ten minutes, and

again in from twenty-five to forty minutes, for collection in or about the tube.

The following substances made up in sea water were used in the concentrations noted:

Substance.	Concentrations.				
Lactic acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Malic acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Butyric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Citric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Potassium oxalate ($K_2C_2O_4$)	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ethyl butyrate			0.08%,	0.008%	
Urea	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Sodium potassium tartrate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ethyl alcohol	95%,	9.5%,	0.95%,	0.095%	
Cane sugar	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Peptone	2%,	0.2%,	0.02%,	0.002%	
Potassium hydroxide	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*,	0.0001 *Mol*
Hydrochloric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*,	0.0001 *Mol*
Potassium nitrate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Diabasic potassium phosphate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Potassium iodide		0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ammonium sulphate		0.6 *Mol*,	0.06 *Mol*,	0.006 *Mol*,	0.0006 *Mol*
Hydrogen peroxide	3%,	0.3%,	0.03%		
Sodium sulphate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	

No reactions which could be attributed to chemotaxy were observed with any of the above substances except the acids.

It was found that with a molecular solution of the organic acids in the capillary tube the spermatozoids were killed in the course of a few minutes. Where 0.1 *Mol* acid was used there was in some cases a collection of spermatozoids around the mouth of the tube apparently attached to the cover glass or slide by one cilium and these vibrated slowly. This collection in a few cases was visible to the naked eye after an hour as a yellow halo about the mouth of the tube. With 0.01 *Mol* solution in some of the trials there was a collection of the spermatozoids in the tube after 45 minutes or more; 0.001 *Mol* had no effect. The accumulation around the mouth of the tube containing 0.1 *Mol* acid and the collection in the tube containing 0.01 *Mol* acid only occurred when sea water was used in which the spermatozoids were very active and numerous.

Such results might be interpreted as cases of chemotaxy.

Kusano (3) has described similar reactions of the swarmspores of Myxomycetes to acids. He attributes them to chemotaxy. An examination of individual spermatozoids, however, showed no change in the direction of their movements passing the mouth of the tube, the effects could by no means always be obtained, and much the same result was observed in one case with HCl. I believe that the results could be interpreted as a toxic phenomenon as explained below.

In the case of the molecular concentration, the diffusing acid is sufficiently strong to kill or paralyze the spermatozoids throughout the whole mount. With acid of 0.1 *Mol* concentration the diffusing zone of the acid which is toxic is not so wide as in the case of acid of the molecular concentration and only those spermatozoids are rendered non-motile which pass comparatively close to the mouth of the tube; hence the collection observed after a time around the mouth of the tube. At the concentration of 0.01 *Mol* the diffusing acid is not toxic, and only those spermatozoids are rendered non-motile which pass comparatively close to the mouth of the tube; hence the collection observed after a time around the mouth of the tube. At the concentration of 0.01 *Mol* the diffusing acid is not toxic, and only those spermatozoids which enter the tube, as some must from several hundred of individuals in a mount, are paralyzed and remain there. Acid of 0.001 *Mol* concentration is below the toxic concentration and we have no effect. We do not find the effects noted above in every mount where the acids are used because there may be an accumulation of all active spermatozoids due to negative phototaxy on the side away from the source of light, in which case they do not meet the acid diffusing from the tube; or there may be too few individuals in the mount to show distinct collection; or they may not be actively motile due to the high temperature or old material.

If the results noted are due to a toxic effect of the acids on the spermatozoids, it would be expected that other substances, such as potassium hydroxide and ethyl alcohol should also produce the reaction. While no response to those substances was noticed, this may have been due to poor material or to too high a temperature at the time of the experiment. In either case the spermato-

zoids would be less active and no accumulation would occur. At the time the work was done the relation between toxicity and chemotaxy was not considered and further work on this phase was not attempted. It would appear that the subject should be investigated.

Experiments on Phototaxy.

That *Fucus* spermatozoids are negatively phototactic is very evident. Under the microscope the large majority will be observed to swim away from the light and in a short time practically all the active spermatozoids will be found on the side away from the light. If a capillary tube lies parallel to the window the spermatozoids in the drop will collect against the tube on the window side, blocked in their movement away from the light. If mounted on a slide containing a bright spot of light in a dark field, there is no collection in the bright area as Englemann (4) found for *Euglena* under the same conditions. The spermatozoids pass in and out of the bright area with no apparent response.

A slide holding a drop of sea water one fourth inch in diameter was placed about two feet from a north window. In less than five minutes practically all the active spermatozoids were found crowded on that side of the drop away from the window. It seemed possible this might be due to gravity.

Two slides were arranged as above, one inclined toward the window and one tilted away from it. In both cases the spermatozoids were found crowded on the side away from the window. In both cases the spermatozoids swam away from the light, but in one case they swam with gravity and in the other against gravity. A liter beaker half filled with sea water yellow with spematozoids was set on a shelf ten feet from a north window. In ten minutes far more active spermatozoids were found in a drop taken from the side away from the window than were found on the lighted side.

Influence of Temperature on Activity.

From indications during the experiments on chemotaxy it was observed that temperature had a very considerable effect on the activity of the *Fucus* sperm and that the optimum temperature is probably relatively low. The experiment below is suggestive.

Sea water yellow with sperm was placed in two vials. One vial was placed in an ice-water bath at approximately 4° C. and the other was left at room temperature of 25°–27° C. Portions were removed from time to time and examined for activity.

Begun at 11:00 A.M.

Hours.	Time.	4° C.	25°–27° C.
½	11:30	More active than at 27°.	Active.
1	12:00	Many active.	Few active.
1½	12:30	Many active.	Few active.
2¼	1:15	Many active.	One or more in microscope field moving slowly.
3	2:00	Many active.	One or more in microscope field moving slowly.
4	3:00	Many active.	One or more in microscope field moving slowly.

Summary.

Using the Pfeffer capillary tube method of determining chemotaxy, it was found that certain acids cause collection of *Fucus* spermatozoids. It is suggested that this may be explained as due to toxicity and not chemotaxy. *Fucus* spermatozoids are negatively phototactic. Low temperature is favorable to the activity of *Fucus* spermatozoids.

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